IN-VITRO DRUG SENSITIVITY OF *RHODOCOCCUS EQUI* ISOLATED FROM NORMAL MICRO-FLORA OF SKIN AND UPPER RESPIRATORY TRACT OF EQUINES

Kishore Kumar[1], S. Maherchandani[2], S. K. Kashyap[3] and D.L. Bohara[4]
Department of Veterinary Microbiology and Biotechnology, College of Veterinary and Animal Science
Rajasthan University of Veterinary and Animal Sciences, Bikaner- 334 001, Rajasthan

Received Revised on: 19.04.2018
Accepted on: 20.07.2018

ABSTRACT

Rhodococcus equi is an important animal pathogen with equines being the most susceptible host. This facultative intracellular pathogen is quite stable in nature and contaminates equine environments. The present study was aimed to study its antibiogram. Out of 202 samples processed 37 (49.33%) isolates were found to be *R. equi*. All the 37 isolates of *R. equi* were subjected to resistotyping with 16 different antibiotics. All isolates of *R. equi* showed 100 per cent sensitivity to Gentamicin followed by ciprofloxacin and streptomycin (sensitivity-97.30%) while higher resistance *i.e.* 97.30 per cent to furazolidone and 59.46 per cent to sulphadiazine was observed.

Introduction

R. equi is facultative intracellular pathogen mainly causing pyogranulomatous pneumonia, ulcerative typhlocolitis, mesenteric lymphadenitis, osteomyelitis, purulent arthritis, reactive arthritis, and ulcerative lymphangitis in foals (Zink *et al.,* 1987 ad Prescott, 1991). This Gram positive organism is responsible for approximately 3% of global foal mortality (Costa *et al.*, 2006). *R. equi* can infect many other farm animals (cats, goats, pigs, cattle and camels) and human beings also. The infections caused by *R. equi* are mostly subclinical for a long period of time. When the symptoms are expressed the foals become untreatable. *R. equi* is an intracellular pathogen surviving and replicating in macrophages and causing granulomatous lesions with thick caseous material. Acquired resistance among *R. equi* isolates has been reported with multiple antibiotics, including doxycycline, penicillin, erythromycin, vancomycin, co-trimoxazole and rifampin (Fierer *et al*., 1987; Verville *et al*., 1994 and Farina *et al*., 1997; Liu *et al.,* 2014).

Therefore, prolonged treatment with judicious choice of antibiotics, probably in combination is required for effective treatment and to avoid relapse of infection (Mascellino *et al*., 1994). It has been seen that with appropriate use of antibiotics survival rate of sick foals can be increased from 20% to nearly 90% (Hillidge, 1987). The present study was conducted to study antibiogram of *R. equi* in apparently healthy animals in the arid region of Bikaner.

Materials and Methods

A total of 202 samples from skin and upper respiratory tract of apparently healthy horses were collected using sterilized swabs and placed in test tubes containing nutrient broth. Out of 202 samples processed for the present investigation 132 were from the non organized farms and 70 were from animals of organized farm. These samples were subjected to culturing for isolation and 37 *R. equi* isolates were obtained. The organisms were isolated and identified as described by Buchanan and Gibbons (1974); Cowan and Steel (1974); Balow's *et al*. (1992) and Quinn *et al*. (1994a). Isolates were confirmed as *R. equi* by 16S rRNA gene based identification carried out as per the method described by Sellon *et al.* (1997).

The antibiotic sensitivity pattern of bacterial isolates was determined by disc diffusion method as per technique of Bauer *et al*. (1966).The results were recorded by measuring the diameter of clear zone of inhibition around each antibiotic disc and compared with the standard zones to assess the status of the isolate in terms of sensitivity.

Results and Discussion

All the 37 isolates of *R. equi* were subjected to resistotyping with 16 different antibiotic and the results were interpreted according to the instructions of manufacturer. The response of organisms was interpreted as sensitive, intermediate and resistant (Table 1). All the isolates were sensitive to gentamicin, streptomycin, ciprofloxacin, co-trimoxazole, erythromycin, kanamycin, tetracycline, chloramphenicol, polymyxin B and amoxyclave. Among aminoglycosides, gentamicin was found to be the most effective antibiotic against *R. equi* for which 100 per cent isolates were found sensitive followed by streptomycin and kanamycin with 97.30 per cent and 91.89 per cent efficacy, respectively. These results are quite similar to Sweeney *et al.* (1987) for gentamicin, Woolcock and Mutimer (1980) for gentamicin and streptomycin and Nordmann *et al.* (1994) for gentamicin and kanamycin.

The β-lactam agents alone showed poor sensitivity (48.65% for cephotaxime, 45.94% for ampicillin, 24.32% for cephaloridine and 13.51% for penicillin) but when associated with a β-lactamase inhibitor, the sensitivity rose significantly (81.08%) for amoxycillin and clavulanic acid (amoxyclave). These observation are quite similar with the study of McNeil and Brown (1994) and Silva *et al.* (2010) in *R. equi* isolates. Among remaining antibiotics, higher sensitivity *i.e*. among fluoroquinolones 97.30 per cent to ciprofloxacin, 94.59 per cent to co-trimoxazole, among macrolides 91.89% to erythromycin, 89.19 per cent to tetracycline and chloramphenicol and 81.08 to polymyxin B, and higher resistance *i.e.* 97.30 per cent to furazolidone and 59.46 per cent to sulphadiazine was observed

[1]Veterinary Officer, Department of Animal Husbandry, Government of Rajasthan; [2]Professor; [3]Professor and Head; [4]Senior Research Assistant, Animal Nutrition Section, National Research Center on Camel, Jorbeer, Bikaner, Rajasthan.

in *R. equi* isolates. Sweeney *et al.* (1987) and Silva *et al.* (2010) reported almost similar sensitivity to tetracycline and chloramphenicol. No significant difference in antibiotic resistivity patterns were observed in our study when compared to earlier works.

Table 1: Per cent sensitivity of different *R.equi* isolates to different antibiotics.

Antibiotic	Sensitive	Intermediate	Resistant
Ampicillin (A)	17 (45.94%)	6 (16.22%)	14 (37.84%)
Cephotaxime (Ce)	18 (48.65%)	–	19 (51.35%)
Cephaloridine (Cr)	9 (24.32%)	3 (08.11%)	25 (67.57%)
Amoxyclave (Ac)	30 (81.08%)	–	7 (18.92%)
Chloramphenicol (C)	33 (89.19%)	1 (02.70%)	3 (08.11%)
Ciprofloxacin (Cf)	36 (97.30%)	–	1 (02.70%)
Co-trimoxazole (Co)	35 (94.59%)	–	2 (05.41%)
Erythromycin (E)	34 (91.89%)	1 (02.70%)	2 (05.41%)
Furazolidone (Fr)	–	1 (02.70%)	36 (97.30%)
Gentamicin (G)	37 (100%)	–	–
Kanamycin (K)	34 (91.89%)	3 (08.11%)	–
Polymyxin B (Pb)	30 (81.08%)	6 (16.22%)	1 (02.70%)
Tetracycline (T)	33 (89.19%)	3 (08.11%)	1 (02.70%)
Penicillin-G (P)	5 (13.51%)	1 (02.70%)	31 (83.78%)
Streptomycin (S)	36 (97.30%)	–	1 (02.70%)
Sulphadiazine (Sz)	14 (37.84%)	1 (02.70%)	22 (59.46%)

References

Balow's A, Hauser WJ, Herrmann KL, Isenberg HD and Shadomy HJ (1992) Pseudomonas and related genera. In: Gilardi, G.L. (Ed.), Manual of clinical microbiology, 5th ed. American Society for Microbiology, Washington, D.C. pp. 429-441.

Bauer AW, Kirby WM, Sherris JC and Turck M (1966) Antibiotic susceptibility testing by a standardized single disk method. *Tech Bull Regist Med Technol.* **36**(3): 49-52.

Buchanan RE and Gibbons NE (1974) Bergey's manual of determinative bacteriology. The Williams and Wilkins Co., Baltimore. **8**: 747-842.

Costa MM, Machado SA, Krewer CC, Ilha MRS, Graca DL, Guaraldi ALM and Vargas AC (2006) Pathogenicity of *Rhodococcus equi* in mice, isolated from environment, human and horse clinical samples. *Pesq. Vet. Bras.* **26**(3):167-170.

Cowan ST and Steel KJ (1974) In: Cowan and Steel's Manual for the Identification of Medical Bacteria, 2nd Ed. *Cambridge University Press, Cambridge, New York*. pp. 90-93.

Farina C, Ferruzzi S, Mamprin F and Vailati F (1997) *Rhodococcus equi* infection in non-HIV-infected patients. Two case reports and review. *Clin. Microbiol. Infect.* **3**(1): 12-18.

Fierer J, Wolf P, Seed L, Gay T, Noonan K and Haghighi P (1987) Non-pulmonary *Rhodococcus equi* infections in patients with acquired immune deficiency syndrome (AIDS). *J. Clin. Pathol.* **40:** 556-558.

Hillidge CJ (1987) Use of erythromycin-rifampin combination in treatment of *Rhodococcus equi* pneumonia. *Vet. Microbiol.***14:** 215-224.

Liu H, Wang Y, Yan J, Wang C and He H (2014) Appearance of multidrug-resistant virulent *Rhodococcus equi* Clinical isolates Obtained in China. *J. Clin. Microbiol.* **52**: 703

Mascellino MT, Iona E, Ponzo R, Mastroianni CM and Delia S (1994) Infections due to *Rhodococcus equi* in 3 HIV infected patients-microbiological findings and antibiotic susceptibility. *J Clin. Pharmacol. Res.***14:**157-163.

McNeil MM and Brown JM (1994) The medically important aerobic actinomycetes: epidemiology and microbiology. *Clin. Microbiol. Rev.* **7**(3): 357-417.

Nordmann P, Keller M, Espinasse F and Ronco E (1994) Correlation between antibiotic resistance, phage-like particle presence, and virulence in *Rhodococcus equi* human isolates.*J. Clin. Microbiol.* **32**(2): 377- 83.

Prescott JF (1991) *Rhodococcus equi:* an animal and human pathogen. *Clin. Microbiol. Rev.* **4**: 20-4.

Quinn P, Carter ME, Markey BK and Carter GR (1994a) Bacterial Pathogens: Microscopy, culture and identification In: *Clinical veterinary microbiology*. Wolfe Publishing, Mosby-Year Book, Europe Ltd. Lyton House, Tavistock square, London, England. pp. 21-66.

Sellon DC, Walker K, Suyemoto M and Altier C (1997) Nucleic acid amplification for rapid detection of *Rhodococcus equi* in equine blood and tracheal wash fluids. *Am. J. Vet. Res.* **58**(11): 1232-7.

Silva P, Miyata M, Sato DN, Santos AC, Mendes NH and Leite CQ (2010) *Rhodococcus equi* isolation from sputum of patients with suspected tuberculosis. *MemInst. Oswaldo. Cruz*. **105**(2): 199-202.

Sweeney CR, Sweeney RW and Divers TJ (1987) *Rhodococcus equi* pneumonia in 48 foals: response to antimicrobial therapy. *Vet. Microbiol.* **14**(3): 329-36.

Verville T, Huycke MM, Greenfield RA, Fine DP, Kuhls TL and Slater LN (1994) *Rhodococcus equi* infections of humans. *Medicine***. 73:** 119-32.

Woolcock JB and Mutimer MD (1980) *Corynebacterium equi: In-vitro* susceptibility to twenty-six antimicrobial agents. *Antimicrob. Agents Chemother*. **18:** 976-977

Zink MC, Yager JA, Prescott JF and Fernando MA (1987) Electron microscopic investigation of intracellular events after ingestion of *Rhodococcus equi* by foal alveolar macrophages. *Vet. Microbiol.* 14: 295-305.